Mass production of self-rooted *Hevea brasiliensis* industrial clones by tissue culture and nursery methods

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Abstract

Industrial clonal plantations of *Hevea brasiliensis*, more commonly known as rubber tree, have been established for several decades with grafted/budded plants as an alternative to the mass clonal propagation of the mature selected industrial clones on their own roots. Substantial investments have been devoted for many years to the development of tissue culture techniques focussing on somatic embryogenesis and micropropagation by axillary budding to reach this goal but with limited success. The recent mass production of self-rooted *H.brasiliensis* industrial clones by nursery methods provides the opportunity to assess the respective pros and cons of *in vitro* versus nursery vegetative propagation methods for mass producing industrial clones of the rubber tree.

Key words: bud grafting, clonal plantations; *in vitro* culture, micropropagation, rooted cuttings, rubber tree

Introduction

Hevea brasiliensis, of the Euphorbiaceae family, is a diploid (2n=36) monoecious allogamic tree species that can reach more than 40m in height in its natural environment, the Amazon basin forest (Webster and Paardekooper 1989). Its rhythmic growth, endogenously controlled, follows Rauh's architectural model (Hallé and Martin 1968; Combe and du Plessix 1974). Its root system consists of one vigorous vertical taproot and several lateral roots (Compagnon 1986).

H. brasiliensis is the main source of natural rubber, which has warranted the domestication of the species since the XIXth century. The first rubber tree plantations were established in Ceylon in 1876 just after the second industrial revolution with seedlings from the Wickham collection in the Brazilian amazon basin forest (Compagnon 1986; Baulkwill 1989). Suitable growing conditions are characterized by annual rainfalls of 1,800 to 2,500 mm with an optional drier season which should not be too pronounced. Soils, slightly acidic, must be well drained as the species is sensitive to waterlogging, root diseases and diebacks (Baulkwill 1989; Watson 1989).

At the beginning of the XXth century, a program of genetic selection was initiated by Dutch researchers in Java and Sumatra from this Wickham seedling population (Baulkwill 1989; Simmonds 1989). Clonal propagation of these materials by rooted cuttings and by grafting techniques was attempted. Only the grafting of hardwood or softwood buds onto seedling rootstock in active growth, developed in 1918 by Van Helten, was efficient enough to be used operationally for selecting and then mass planting the rubber tree clones (Dijkman, 1951). In the 1930's, the superiority of industrial rubber tree clonal plantations over seedling-derived ones was definitely acknowledged (Compagnon 1986; Dijkman, 1951).

Rubber tree clonal plantations are usually planted in monoclonal blocks of budded trees that grow upon a genetically heterogeneous population of seedlings rootstocks (Clément Demange et al. 2007). Despite a much higher and uniform productivity than seedling-established plantations, grafted clones are exposed to:

i. Graft-induced drawbacks encompassing imbalanced aerial development as well as various kinds of scion X rootstock incompatibilities which can be one cause of within-clone variability and of the bark necrosis syndrome (Masson and Monteuuis 2017).

ii. Maturation-induced negative effects of the material produced by conventional budwood gardens (Masson and Monteuuis 2017).

Rationale and historical background of producing *H. brasiliensis* self-rooted clones

As early as in the 1910's, researchers observed that the grafted clones failed to perform as well as the original mother trees they derived from (Compagnon 1986). Further experiments (Dijkman 1951) demonstrated that cutting-derived clones were more vigorous, less prone to early production of lateral and axillary shoots, and yielded more latex than the same clones produced by grafting.

Graft-induced negative effects were assumed to be the cause of such differences. The production of the same clones on their own roots appeared logically as the most rationale way of investigating further this issue.

Rooting *H. brasiliensis* cuttings was attempted at the end of the XIXth century using softwood terminal shoots averaging 30cm in length but these shoots, especially when collected from mature materials, died before they could be rooted (Warburg 1902). This situation remained unchanged for nearly 50 years till Stahel (1947) improved significantly the rooting rates by using permanent fine water sprays. Soon after that, Dijkman (1951) succeeded in rooting shoots taken from the crown of 3 to 4 year-old seedlings that were placed in open air rooting beds under a similar water misting system. The adventitious roots formed looked like tap roots, which was considered as a promising indicator of suitability for field planting. Levandowsky (1959) became the first to root mature material cuttings under an open air intermittent mist-system in nursery conditions. This technique was soon after applied successfully with up to 90% of rooting rates to fifty mature rubber trees including representatives of industrial clones PB86 and GT1, the most widely planted genotypes in Malaysia at that time (Tinley and Garner 1960). Surprisingly these activities were not pursued leading Compagnon (1986) in the mid 80's to state that none of the numerous attempts to mass propagate industrial rubber clones cost-effectively by rooted cuttings had really succeeded so far. From the 70's, attempts to produce self-rooted rubber trees by cuttings were gradually abandoned to give preference to tissue culture that was actively booming at that time.

Background, prospects and current limitations of the tissue culture option

In vitro micropropagation by axillary budding of *H. brasiliensis* has been initiated using shoot apices or shoot nodes as primary explants preferably because shoot apical meristems are too tiny in this species to be successfully inoculated (Venkatachalam et al. 2007; Monteuuis, unpublished results). Protocols developed since long ago for *in vitro* multiplying and rooting *H. brasiliensis* microshoots have been reported to be not efficient enough for mass producing industrial rubber tree clones (Lardet et al. 1987). Despite substantial investments during the past 40 years this situation has not radically improved lately (Venkatachalam et al. 2007). Moreover, the microshoots rooted *in vitro* cannot be easily acclimatized (**Fig. 1**) to natural environment to be planted ultimately, especially when the tissue culture facilities are distant from the nurseries and the planting sites (Dibi et al. 2010; Masson 2017).

Figure 1: Transfer in SoGB nursery facilities of *H. brasiliensis* microcuttings produced *in vitro* in Europe and sent under proper conditioning 4 days before.

Somatic embryogenesis was first attempted on rubber trees at the Rubber Research Institute of Sri Lanka in 1972 using anther-derived calli from which embryoids then shoots were obtained a few years later (Satchutanantavale and Irugalbandara 1972; Paranjothy and Gandimathi 1975). Subsequent works have established that *H. brasiliensis* is one of the few woody species for which somatic embryogenesis can be obtained from sporophytic tissues - mainly from immature anthers, seed integuments and roots - of mature trees (Carron et al. 1989; 1995). However, the success rates are still very low and strongly genotype-dependent, irrespective of field performance. Thus it can be assumed that only about 20 of the 50 industrial rubber tree clones planted worldwide have successfully responded to somatic embryogenesis initiation so far with a much lower proportion being able to fully develop into somatic embryos. These are prone to somaclonal variation risks liable to vary according to the genotypes and the protocols used, somatic embryogenesis procedures maintained long-term being more exposed (Compagnon 1986; Montoro et al. 2012). Moreover only small quantities of somatic embryos can be produced using the procedures developed so far, but their numbers can be amplified by micropropagation by axillary budding, notwithstanding the foregoing limitations of this technique. Because of this very low efficiency, it is estimated that a ready for planting *in vitro*-issued rubber tree costs nowadays 30 times more than the same material traditionally produced by grafting.

As regards field performance, a few observations indicated that self-rooted tissue culture plants had a better growth and a higher latex production than grafts of the same age and of the same genotype (Dibi et al. 2010; Montoro et al. 2012). However, these statements were based on insufficient numbers of plants and of clones assessed during a too short period for drawing definitive conclusions regarding the superiority of self-rooted vs grafted materials. Besides, between clone-differences may exist and the clones that are best in West Africa conditions like PB217 and IRCA331 can still not be produced by tissue culture (Carron et al. 2009; Masson unpublished results).

Prospects and current limitations of the rooted cutting option

These *in vitro* limitations spurred the Société des caoutchoucs de Grand Béréby (SoGB) in the Ivory Coast to revive the experiments on the production of industrial rubber tree clones by rooted cuttings in local nursery conditions. Soon after, SoGB was able to root several hundred cuttings of two mature clones with a 75% success rate, benefiting from good nursery facilities equipped with a reliable mist system (Masson et al. 2013, **Fig. 2**). Since 2013, the technique has kept improving and all the 36 industrial clones tested so far could be rooted by the SoGB, especially the best ones, PB217 (**Fig. 3** and **4**) and IRCA331, which could be considered as a meaningful follow up of the work initiated by Levandowsky in the 50's. Although the best rooting scores were obtained from *in vitro*-derived plants, suitably managed as responsive stock plants, it has to be mentioned that average rooting rates of 60% were recorded for shoots collected from field growing mature industrial clones. The first observations tend to indicate that adventitious rooting capacity varies from one mature clone to another. Efforts are being pursued to check if these clonal differences cannot be lessened by applying rejuvenating practises to the stock plants. Such physiological conditioning is also expected to increase the number of shoots with a high rooting capacity in order to produce more rooted cuttings in shorter delays for greater overall efficiency.

The SoGB experience shows that industrial *H. brasiliensis* clones can be self-rooted by rooted cuttings in local nursery conditions at a much cheaper cost and for a greater number of clones than by tissue culture. Nursery-produced rooted cuttings are also more robust, adapted to natural conditions and can thus be more successfully field planted.

Figure 2: Rooting cuttings of *H.brasiliensis* industrial clones in SoGB nursery facilities.

Figure 3: Three-month-old container-grown rooted cutting of clone PB217 at SoGB.

Figure 4: Root system of a 3 month-old rooted cutting of clone PB217 at SoGB.

Rationale of combining tissue culture and nursery techniques for mass producing self-rooted *H. brasiliensis* industrial clones

Although first attempted with disappointing results, the possibility of mass producing *H. brasiliensis* clones by rooted cuttings has soon be abandoned in favor of grafting which has rapidly been adopted as an alternative and more efficient way for developing, on a large scale, industrial clonal plantations of rubber trees (Dijkman 1951; Webster and Baulkwill 1989). Grafted *H. brasiliensis* clones however combine advantages but also drawbacks (Masson and Monteuuis 2017), and the prospects of field testing clones on their own roots have remained a prevailing objective at the research and development levels (Dijkman 1951; Compagnon 1986; Webster 1989). During the past few decades, substantial efforts have been devoted to tissue culture for achieving this goal while nursery techniques have been neglected untill SoGB decided recently to reconsider their usefulness and demonstrate their efficiency (Masson et al. 2013). Although the tissue culture and nursery protocols currently available are very likely amendable, it is warranted to consider whether nursery and *in vitro* vegetative propagation methods can complement each other synergistically for greater overall efficiency, as has already been demonstrated for other tree species (Thompson 2014). Tissue culture could be useful for vegetatively multiplying selected genotypes with shorter delays and in bigger amounts than in nursery. The microshoots produced *in vitro* can be rooted preferably in *ex-vitro* conditions, as is routinely practiced, with great success, with teak for instance (Goh and Monteuuis 2016).

Adapted *in vitro* protocols can help also for physiologically rejuvenating the mature selected clones in a more efficient way than in nursery conditions by miniaturizing the sporophytic tissues used. In this respect, the possibility to get somatic embryos from mature selected genotypes on *H. brasiliensis* is real advantage. However, the rejuvenation achieved by somatic embryogenesis, although undoubtedly recognized as complete from an ontogenetical standpoint, may be more questionable physiologically as possible negative ageing influence of non-optimal medium components on tissue cultured soft and permeable isolated cells or group of cells cannot be ruled out (von Aderkas and Bonga 2000).

Rubber tree tissue culture has been for a long period of time disconnected from the trees in their natural environment, far away from most of *in vitro* facilities. Closer connections and location proximities between field, nursery and tissue culture activities should be encouraged for introducing plant material that can be physiologically preconditioned for greater culture initiation success (Monteuuis et al. 2011). Deeper knowledge of *H. brasiliensis* physiology in natural environment may also help in simplifying tissue culture protocols to make them more operational and cost effective. In this respect, benefitting downstream from nearby nursery facilities must be considered as a real asset for the *ex-vitro* rooting and acclimatization process, as demonstrated for teak for instance (Monteuuis 2000; 2016).

Conclusion

The recent progress, especially in nursery techniques, made by SoGB have promoted the mass production of industrial rubber tree clones on their own roots, with particular mention for the outstanding clones PB217 and IRCA331 which could not be produced by tissue culture so far. This has given the possibility to set up well–designed with big enough sample size trials to compare reliably the field performances of self-rooted vs grafted *H. brasiliensis* clones (**Fig. 5**). Efforts are pursuing along this line in SoGB on an increasing number of industrial clones in order to take into account possible clone X clonal propagation technique interactions. These activities are expected to soon provide a definite answer to the old question of knowing whether latex yield can be significantly increased using clones on their own roots instead of grafts. In the affirmative, the future of self-rooted rubber-tree clonal plantations will then be ultimately dependent on economic aspects. In this regard , the possibility to improve the efficiency of tissue culture and nursery techniques separately or combined for mass producing self-rooted *H. brasiliensis* industrial clones will have a major impact.

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Figure 5: Five-month-old rooted cuttings of clone IRCA331 field tested at SoGB.

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